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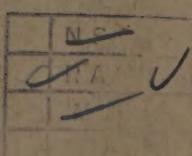
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Report to the
Government of

INDIA

THE SANDAL SPIKE
DISEASE

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
ROME, 1960



REPORT

to the

GOVERNMENT OF INDIA

on

THE SANDAL SPIKE DISEASE

by

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Rome, 1960

INTRODUCTION AND ACKNOWLEDGEMENTS	1
SUMMARY	2
I. THE SANDAL TREE (<u>Santalum album</u> L.)	4
II. SURVEY OF THE LITERATURE ON SANDAL SPIKE DISEASE	6
III. DESCRIPTION OF SANDAL SPIKE DISEASE	12
A. Symptoms	12
B. Transmission	13
C. Control Measures so far Recommended	13
(a) Removal or killing of spiked sandal trees	14
(b) Removal of <u>Lantana camara</u>	14
(c) Selection of healthy, resistant mother-trees for seed production	14
(d) Use of resistance-inducing host plants	14
IV. PROPOSALS FOR FURTHER WORK ON SPIKE DISEASE	16
A. Diagnostic Work	16
1. Indexing of various test plants	16
(a) Mechanical sap inoculation	16
(b) Grafting methods, etc.	16
(c) Dodder transmission	16
2. Serological work	17
(a) Production of antiserum	17
(b) Absorption of antiserum	19
(c) Use of antiserum	20
B. Transmission Studies	20
1. Grafting transmission	20
2. Insect transmission tests	21
3. Soil and seed transmission tests	21
C. Control Measures	22
1. Removal of sources of infection	22
2. Protection against possible vectors	22
3. Selection and testing of apparently resistant mother-trees of sandal	22
(a) Selection	22
(b) Testing for virus infection	22
(c) Testing for resistance	23

(Continued.)

	<u>Page</u>
4. Selection and testing of suitable host plants	23
(a) Inoculation of experimental plants	23
(b) Testing of experimental plants	23
(c) Repetition of inoculations	23
5. Vegetative propagation (by cuttings in mist propagation) of virus-free sandal trees and their most important host plants	24
(a) Cuttings from young shoots	24
(b) Cuttings from mature shoots	24
6. Thermotherapeutic treatments	24
(a) Experiments	24
(b) Testing of treated material	24
V. RECOMMENDATIONS CONCERNING STAFF, FACILITIES AND EQUIPMENT	26
A. Staff	26
B. Facilities	26
C. Equipment	27
REFERENCES	28
ILLUSTRATIONS	32

INTRODUCTION AND ACKNOWLEDGEMENTS

In accordance with the technical assistance agreement between the Government of India and the Food and Agriculture Organization of the United Nations, an expert was sent to India "to study the problem of sandalwood spike disease in detail and to lay down a phased program of investigation, aimed at (a) immediate remedial steps to control the disease, and (b) long-term studies to eradicate the disease or to develop immunity in the tree species".

The expert, Mr. H. Rønde Kristensen, visited India during the period 27 September 1959 - 27 January 1960. Acknowledgement is due to the Ministry of Agriculture in New Delhi, and the Minister of Forestry of Mysore State, for the interest taken in the assignment.

The first fortnight of October was spent at the Forest Research Institute at Dehra Dun. From 15 October 1959 - 22 January 1960 the writer's headquarters was at the Forest Research Laboratory, Bangalore. During the stay in Bangalore, excursions were made to various sandal areas in the States of Mysore and Madras and visits were paid to Research Institutes in Madras, Coorg, Poona and Bombay, as well as in Bangalore. In this way, the writer was able to familiarize himself with the problem of the spike disease as it presents itself today, and with the work done in the past on this important disease. The collection of the necessary information, however, was made possible only through the unfailing assistance and cooperation of many forest officers, research workers, scientists and government officials. Very valuable help and information were received from the chiefs and staff members of the Forest Departments in Mysore and Madras, from the Indian Institute of Science, from the President and staff members of the Forest Research Institute at Dehra Dun, and from the members of the Working Committee on Sandal Spike Disease. In particular, thanks are due to the Chief Research Officer of the Forest Research Laboratory at Bangalore, Dr. M. N. Ramaswamy, for his outstanding and kind assistance and collaboration on every matter throughout the assignment.

SUMMARY

Spike disease is a very serious virus disease of the sandal tree (Santalum album Linn.).

The disease was first reported in Coorg in South India in 1899 and has since spread to many forest districts of the States of Mysore and Madras, where the damage caused has been considerable - indeed some forestry officers and research workers have expressed the view that spike disease now threatens to ruin the whole sandalwood industry, which has hitherto been a monopoly of South India. This danger was already realized many years ago; a number of research workers have been studying the problem and very useful information has been obtained through the years.

In 1917, L. C. Coleman proved the infectious nature of the disease and showed that the spike disease could be transmitted by grafting. In later experiments it was shown that the infectious agent could also be transmitted by budding and by a special leaf insertion method, and, to some extent, through the haustoria of the sandal plant.

Insect transmission tests seem to indicate that one or more species of leaf hoppers can transmit the disease, but it is generally agreed that these experiments should be confirmed by repetition.

The sandal tree is a root parasite and numerous host plants have been registered. These host plants seem to have a great influence on the sandal plant in regard to growth, seeding, heartwood formation, and resistance to insects and spike disease.

Individual sandal trees display differences with regard to resistance to spike disease, and it has several times been reported that healthy-looking sandal trees are found among severely diseased trees. Such trees are therefore considered to be more or less resistant, but so far they have not been actually tested for resistance.

Removal or killing of infected sandal trees (i.e. sources of infection) has in earlier years been recommended and carried out to some extent, but it is claimed that the results have not been satisfactory.

The present report summarizes the work carried out in the past and gives a description of the external symptoms of the disease as well as of the histological, chemical and physiological changes which take place in diseased plants.

In order to arrive at definite recommendations with regard to control measures, more experimental work is needed. Detailed proposals for this work are made under three headings :

- A. Diagnostic work.
- B. Transmission studies.
- C. Control measures.

The diagnostic work comprises indexing to various test plants (infection experiments) by various transmission methods, and, secondly, serological methods. The main purpose is the development of a quick and reliable diagnostic method so that virus-infected plants can easily be detected (including those with latent infection) and sources of infection removed as early as possible.

Among the various kinds of transmission work suggested, most emphasis should be laid upon insect transmission studies, the aim being the identification of the insect vectors of spike disease.

The control measures are much dependent on the work concerning diagnostic and transmission methods.

Removal of sources of infection can first be really effective when all sources within a certain area can be removed, and protection (e.g. spraying with insecticides) against vectors can naturally first be recommended when the virus-vector relationship has been completely established.

Strong emphasis should be laid upon the selection and testing of healthy, resistant mother-plants, both of sandal trees and of the most important host plants, and simultaneously efforts should be made in order to develop a quick and practical method of propagating such plants by cuttings, so that the progeny will also contain resistance genes. If such work is successful, it will in future be possible to replace spike-diseased trees with healthy, resistant ones, and in regular plantations of sandal trees it will perhaps be possible to make use of such resistant trees associated with well-known and resistant host plants.

Recommendations are made with regard to the necessary staff, facilities and equipment. Much emphasis is placed on the use of insect-proof greenhouses for future work on sandal spike so that the investigations suggested can be carried out under controlled conditions.

I. THE SANDAL TREE, SANTALUM ALBUM L.

Santalum album L., the sandalwood tree, is sacred to the Hindus; the tree is described in the Epics of India (the Valmiki Ramayana and the Mahabharata), and it is also mentioned in the old Sanskrit literature. In India, the natural sandal forests are mainly to be found in the States of Mysore and Madras, and only to a small extent outside these areas. Elsewhere, the species has been recorded in China, Cochin China, in parts of Australia and on several islands of the Eastern Archipelago (e.g. Java), and it has been introduced in Africa and Japan.* However, it is claimed that the tree does not produce much scented wood away from its natural habitat in India.

The sandal tree is an evergreen tree with slender, drooping branchlets (R. S. Troup, 1921). The leaves are opposite, sometimes alternate, ovate or ovate-lanceolate. The length of the leaves on healthy trees is 2.5 - 4.5 cm, sometimes larger. Flowering and fruiting often commence at an early stage and have been observed even on 3 - 4 year old trees. The fruit is a stone fruit, and natural as well as artificial regeneration has so far in nearly all cases been by seed.

The heartwood which constitutes the valuable part of the tree, is either yellowish or reddish (depending on the type of sandal) and is strongly scented due to the oil content. The heartwood formation is markedly influenced by environmental factors, but, as a rule, very little heartwood is produced before the tree has reached an age of 25 - 30 years. Foresters usually prefer a sandal tree to reach an age of at least 40 years before being utilized.

One of the most notable features of the sandal tree is the fact that it is a root parasite; this was established in 1871 by John Scott (then Curator of the Royal Botanic Garden, Calcutta). Later (at the beginning of this century), this parasitism was confirmed by C. A. Barber (Government Botanist, Madras), who published a list of more than 100 different host plants of sandal. Since then, up to about 500 host plants have been recorded, ranging from forest trees to herbaceous weeds. Generally, it is claimed that a young sandal seedling cannot survive more than one year without being attached to a host plant. There seems, however, to be evidence to the effect that an old tree is able to exist for several years without any visible host plant in its neighbourhood. So far, no systematic investigation, under controlled conditions, has been carried out of the sandal tree's dependence on host plants. As practically all sandal trees are attached to one or another host plant, it is obvious that any research work on the sandal tree must take due regard of the various host plants which undoubtedly exercise considerable influence on the health and growth (formation of heartwood, etc.) of the sandal tree.

* Information from the Forest Research Institute, Dehra Dun.

Under Indian conditions, the sandal tree thrives well from sea level up to about 1,200 m altitude, but the formation of heartwood seems to be best between 600 and 900 m, and with an annual rainfall of 850 - 1,350 mm. Thus, a comparatively cool climate with moderate rainfall, much sunshine and long periods of dry weather suits the species best.* In South India, its native habitat comprises an area of about 125,000 km². The bulk of the trees occur in open scrub jungles; it is sparsely distributed in pole forests and rarely seen in high forests. Sandal trees are also found extensively on cultivated lands, private holdings, and village sites (M. A. Muthana, 1955).

In Mysore State, the sandal tree is declared a "royal tree", i.e. its utilization is an exclusive government monopoly wherever it is found, and strict rules govern the felling, dressing, transport, preparation and sale of sandalwood and its products. In Madras State, where sandal is not a "royal tree", the major supply of wood comes from the forest reserves. The average price of sandal heartwood -- perhaps the most valuable wood in the world -- is about Rs. 5,000 per ton. The wood is mainly used for the distillation of its oil (highest content in roots), which is used for perfume, soap, and for medical purposes. To some extent the wood is also used for carving and other decorative work, as well as in cremation ceremonies.

The economic importance of sandal may be judged from the fact that the revenue to the States from sandalwood oil during the period 1949/50 - 1954/55 amounted to 2.5 crores of rupees, or 5 million rupees annually (A.V.V. Iyengar, 1958).

* Information from the Forest Research Institute, Dehra Dun.

II. SURVEY OF THE LITERATURE ON SANDAL SPIKE DISEASE

The first detailed description of the disease appears in a memorandum submitted in December 1899 by C. McCarthy (then Deputy Conservator of Forests in Coorg) to the Secretary to the Chief Commissioner of Coorg. It is, however, most likely that the disease - like many others - had been in existence several years before it was first reported. McCarthy was of the opinion that "something in the nature of a ferment is communicated to the sap of diseased trees". Since McCarthy wrote his memorandum, several authors have contributed to the vast number of reports which have been published on the subject, and many different theories as to the cause of the disease have been put forward.

E. J. Butler (1903) considers spike to be a nutritional disease characterized by a forced carbon-assimilation which may be due to the circulation of a poison in the sap.

C. A. Barber (1903) concludes that the disease is due to the death of the root ends of the tree, but he does not find it likely that any bacterial disease is involved. Barber thinks it most probable that the disease is spread through the roots; he emphasizes the serious character of the disease.

L. C. Coleman (1917) carried out various experiments and proved spike disease to be transmittable by grafting. He states that "sandal spike is a very serious disease which has already (1917) spread over large portions of the sandal-growing area of South India". He further expresses the opinion that the possibilities of damage from spike disease in the future are enormous, and his conclusion is that the disease is caused by a virus which in some cases may be spread by insects.

R. S. Hole (1917) advances the theory that spike disease is provoked by "an unbalanced circulation of sap" which may be caused by a number of different factors such as fire, death or damage of sandal host plants, partial suppression of sandal by Lantana or other plants, or exposure of sandal trees hitherto growing under shade. Additional factors which may cause spike are drought, bad soil-aeration and damage by insects. Hole does not believe that spike disease is really infectious.

C. M. Hodgson (1918) is compelled to resort to the virus theory and he is convinced that spike is not caused by fire or any other agency of the environment. He believes that the disease is seed-borne, and points out that spike is very rare on trees in old ploughed fields still under cultivation.

P. M. Lushington (1918) is strongly opposed to the theory of "unbalanced circulation of sap". According to investigations made, seed transmission of spike is not likely to take place.

C.E.C. Fisher (1918) states that "the one theory that seems to fit in with the facts known at present is that spike is caused by bacterial agency by what is known as ultra-microscopic organisms".

L. C. Coleman (1923) reports on confirmation of the transmissibility of spike disease by grafting, and he proves that haustorial transmission can also take place, but "it appears to me unlikely, if not impossible, that the natural transmission of spike of sandal takes place only through the haustoria". Coleman again indicates the possibility of insect transmission.

W. C. Hart and S. Rengaswamy (1926) estimate the loss caused by sandal spike disease during the period 1917-25 at Rs. 4,700,000 in the North Salem Division alone. In their publication it is also indicated that soil transmission does not seem to take place, and that sandal did not become infected when grafted with spiked plants of Zizyphus or vice versa.

M. Sreenivasaya and G. Gopalaswam Naidu (1928) succeeded in transmitting spike disease by budding; they claim that by this technique transmission of spike can be effected in young sandal plants 6-9 months old and that in their experiments infection took place 131 days after budding.

M. J. Narasimhan (1928), through cytological investigations, proved intra-cellular inclusions to be present in the cells of spike-infected leaves. Such inclusions have never been found in healthy leaves.

M. Sreenivasaya (1930) reports on new and improved transmission methods. The "leaf insertion method" in which parts of fresh, diseased leaves are inserted between the wood and the bark of the healthy plants seems especially promising. Up to 72 per cent transmission has been obtained by this method, the symptoms appearing in the inoculated plants 45-60 days after the insertion. Sap transmission tests, on the other hand, yield negative results. There exist several varieties of sandal which differ with regard to disease-resisting properties; one very resistant variety has a high haustorizing capacity.

M. Sreenivasaya (1933(a)) points out the importance of the removal of sources of infection and, in order to detect disease-masking sandal trees, he strongly recommends the pollarding of all suspected trees, which after such treatment will show any symptoms in the new flush of growth.

M. Sreenivasaya (1933(b)) states that two types of resistance exist among sandal plants: (1) autogenic resistance, which is exhibited by certain plants and which is largely independent of the nature of the associated host plant, and (2) acquired resistance, which is built up by the tree if certain species of hosts and certain conditions of environment are provided.

M. J. Narasimhan (1933) found that spike disease stimulates the formation and produces an excess of starch grains in the chloroplasts.

Anonymous, "Nature" (1933) : The insect-borne nature of spike disease is claimed to have been established through entomological work conducted by the Forest Research Institute, Dehra Dun. Transmission with the leaf hopper Moona albimaculata yielded three positive results. In experiments with aphids, one case is suspected to be positive, but even if aphids can act as vectors they are not believed to be of any practical importance as they are said to be very rare on sandal trees.

M. G. Venkata Rao and K. Gopalaiyengar (1934(a)) describe two types of spike disease, the rosette type (the common type) and the pendulous type (the new type).

M. G. Venkata Rao and K. Gopalaiyengar (1934(b)) suspect that, unlike other similar virus diseases, a minimum of the infective agent may be necessary to transmit the disease. For that reason, twig-grafting would be preferable to any other transmission method.

S. Rangaswami and M. Sreenivasaya (1935), in summarizing results of platform experiments, declare that spike disease is insect-borne and that the insect vectors are active during the night; among 265 types used in mass transmission tests 8 types may be suspected as vectors.

A.V.V. Iyengar and S. Rangaswami (1935(a)) claim that it has been possible to control the spread of spike disease through effective and thorough removal of diseased plants.

A.V.V. Iyengar and S. Rangaswami (1935(b)), as a preliminary result of their studies of the use of various plant poisons, state that arsenicals seem to be best for killing off the majority of affected trees in less than four weeks. The action of these chemicals, however, is influenced by the season and the stage of the disease.

M. G. Venkata Rao (1935) points out the importance of undergrowth in the spread of spike disease. The incidence of disease is highest in scrub jungles and lowest on cultivated land in private holdings. Among the undergrowth, Lantana in particular is suspected, but this is not the only plant responsible for the spread of spike disease. Very young sandal plants growing under bushes were found to be without spike; the disease did not appear until the sandal was above the bushes.

A.V.V. Iyengar (1938(a)) describes various aspects of control of spike disease and claims that the removal and destruction of diseased sandal trees - which has been carried out on a large scale - has not had sufficient effect on the spread of the disease. He also states that so far no host plant has induced complete immunity to the spike disease.

A.V.V. Iyengar (1938(b)) reports on various physiological and physical methods for identification of spike disease. In diseased sandal leaves, the Ca/N ratio is much lower than in healthy leaves. A simpler and more useful

technique for practical purposes involves biometric measurements on leaves and twigs, based on the fact that the ratio of length to breadth of the affected leaves (L/B) is significantly higher than that of the healthy ones.

M. G. Venkata Rao (1938) : Good and bad hosts of sandal can only be differentiated when grown individually with sandal. Of 108 different host plants which were grown with sandal in pot cultures and tested with artificial inoculation, none could induce resistance to the disease.

S. Rangaswami and A. L. Griffith (1939) : Surveys in spiked areas in patches that had remained healthy for a long time showed great differences in the floristic composition between the healthy patches and the surrounding spiked areas. Infection trials with healthy sandal plants grown in pots together with various host plants showed that some of these possess a very high degree of resistance to spike disease, generally in accordance with the results of the survey in the field.

S. Rangaswami and A. L. Griffith (1941) : In experiments carried out in 1940, the leaf hopper Jassus indicus (Walk) proved to be a vector of spike disease. Fifty per cent of the experimental plants developed the disease. The authors claim, however, that the experiment needs confirmation by repetition.

M. Sreenivasaya (1948) : The sandal plant is influenced by its associated host plants with regard to :

- (1) growth;
- (2) seeding;
- (3) heartwood formation;
- (4) resistance to insect attack and spike disease.

While infection by grafting is easy to carry out in some places, it has been a complete failure in other areas, and this would be explained by differences in the floristic composition. The actual infection occurs during April/May, and the season for the first external symptoms to appear after infection is the following year in April/June. Removal of diseased sandal trees is strongly recommended.

M. J. Narasimhan (1954) : Cytological studies have shown that starch grains were large and abundant in the pith and medullary rays of the stems and petioles, but minute in the chloroplasts of diseased leaves, and that phloem necrosis was marked by collapse of sieve-tubes.

A.V.V. Iyengar (1955) : Investigations have revealed that roots of spiked sandal plants contain high quantities of lime and only small amounts of nitrogen.

E. A. Larsrado (1955) : It is concluded that all control measures yet attempted have been unsuccessful. The development of resistant

varieties would appear to be the most promising line for future research.

M. A. Muthana (1955) gives a review of the spread of spike disease up to 1950 :

1899	:	Approx.	207	km ²
1926	:	"	5,535	"
1934	:	"	14,800	"
1949	:	"	18,355	"
1950	:	"	18,390	"

The author also summarizes the measures taken to combat spike disease in Mysore State.

1893 : A mysterious disease was found to have attacked sandal trees in Coorg.

1903 : Investigations started in Coorg (Barber and Butler).

1903/4 to 1918 : 700,000 infected sandal trees were burnt without any beneficial results.

1907 : The Government of Mysore offered a reward of Rs. 10,000 for discovery of the disease.

1917 : Transmission by grafting was proved by L. C. Coleman.

1917 to 1928 : Sporadic work carried out.

1928 to 1934 : Systematic investigation conducted by both Mysore and Madras Governments employing special staff (Coleman, Narasimhan, Venkata Rao) :

- (1) Host plant's influence.
- (2) Two types of resistance.
- (3) Influence of shade on susceptibility.
- (4) Season of symptom expression May/June.
- (5) More grafting transmission.

1934 to 1947 : Special staff employed ceased to function in 1934 and further work was mainly concentrated on killing diseased trees in selected border areas. The number of trees killed from January 1935 to the end of December 1936 was 51,087, but all this removal did not have the desired effect as the disease spread beyond these areas.

Sporadic laboratory work :

(a) Introduction of so-called "spike-resistant" hosts to minimize susceptibility.

(b) Serological experiments (negative results).

(c) Dosing with trace elements such as zinc, boron, etc. (negative results).

1948-55 : A special Sandal Spike Committee was formed, and at its meeting on 4 June 1949 it recommended the establishment of a working committee. On 18 January 1950, the Government sanctioned the formation of such a working committee. The following field and laboratory program was suggested :

Field work :

- (1) Raising stock of sandal with selected host for use in pot culture experiments.
- (2) Experiments concerning the influence of various hosts on the susceptibility of sandal.
- (3) Investigation concerning various (exotic) species of Santalum in regard to susceptibility.
- (4) X-ray treatment of sandal seeds (development of resistance).

Laboratory experiments :

- (1) Serological investigations.
- (2) Estimate of Cu in healthy and diseased plants.
- (3) Dosing of sandal with selected chemicals.

The Committee also requested the Government to obtain the services of a virus specialist (through FAO) for six months.

M. A. Muthana concludes his review by saying that the spike disease has so far defied the devoted efforts of some of the best men all these years.

M. N. Ramaswamy (1956) summarizes facts about the sandal spike disease up to date and suggests that emphasis in future investigations should be laid upon diagnostic work, isolation and study of the virus itself, and host plant studies.

III. DESCRIPTION OF THE SANDAL SPIKE DISEASE

Many authors have, in the past, made contributions to the knowledge of the spike disease, but there are still several important gaps to be filled, and some of the results obtained seem to need confirmation. However, it is possible on the basis of the information at present available to give a fairly comprehensive description of the disease. Also, it may be considered safe to conclude from the experimental results so far obtained that spike disease is caused by an agent of a virus nature.

A. Symptoms

The most characteristic symptom of spike disease is the severe reduction of the leaf size and the shortening of the internodes, so that the leaves become too crowded on the leaf-bearing branches (see illustrations). Simultaneously, the leaves show a tendency to stand out stiffly from the branches, which acquire a spike-like appearance. As the disease progresses, the leaves become yellowish, and shortly before the death of the tree - which is the final result of infection - the leaves may get a reddish tinge. The symptoms do not normally appear at once all over the affected sandal tree; most commonly only parts of a tree show the symptoms to start with, and then they gradually spread to the entire tree. Research has demonstrated that the longest period a sandal tree can live after infection is three years, the average period being fifteen months. The flowers of infected trees often show the phenomenon of phyllody, and diseased parts of such trees very rarely bear any fruits, while the symptomless parts of infected trees often flower and fruit extremely well. It has been shown that masking of symptoms of infected trees does sometimes occur, but by pollarding such trees the symptoms will develop in the new flush of growth. The death of infected trees is a natural consequence of the death of the haustoria and root ends - a feature which is another characteristic of the disease. Research has also established that significant histological, chemical and physiological changes take place in the diseased plants, which show a pronounced starch accumulation in the leaves, necrosis of the phloem and intra-cellular bodies associated with the nucleus of the cells. Histidine can be found in large amounts, and mannitol has only been found in infected plants, in the leaves of which there is also an accumulation of oxalic, succinic and malic acid (possibly due to respiratory changes). The enzyme concentration and the osmotic pressure in the cell sap of diseased plants is above normal, while the freezing point is lower. Spike-diseased plants contain lower amounts of calcium and potassium, while the amounts of phosphorus, manganese and nitrogen are higher than in healthy plants. Especially marked differences between the leaves of diseased and healthy plants are found in regard to the content of calcium and nitrogen, so that the ratio Ca/N can be used as a diagnostic means of determining whether or not a plant is infected. The reduced size of diseased leaves is actually presumed to be due to calcium deficiency, which is caused by the poor intake of calcium by the roots, which again is supposed to be due to the effect of some toxins secreted after infection.

The most rapid movement of spike virus so far measured in sandal trees is 30 cm in 89 days after experimental inoculation.

In the field, the disease does not usually appear to affect saplings below 4-5 years of age when they have flowered and fruited at least once. In experiments it has, however, been possible to transmit the disease to 8-10 month old saplings.

B. Transmission

The spike virus can be transmitted by various forms of grafting and budding and infection of healthy trees has been effected by grafting material from trees showing distinct spike symptoms as well as from disease-masking trees. The average period from infection to manifestation of the disease in the case of artificial infection is 4½ months. When pollarding artificially-infected plants, the symptoms will normally appear much more quickly than otherwise. Originally twig grafting was supposed to be the most effective way of transmitting spike virus to healthy plants, but, according to the results of some experiments, transmission takes place just as readily by means of a special leaf insertion method in which part of an infected leaf is inserted between the wood and the bark of the plant to be infected. The highest experimental transmission percentage was obtained by infection in May/June, and the poorest results by infection in October. It has been shown that shaded plants are more susceptible to infection than those exposed to full light. The spike virus can also be transmitted by the haustoria, but apparently only with difficulty, so it is not likely that this means of transmission is of any practical importance. Seed transmission of spike virus has not been proved with certainty, neither has soil transmission. Experiments to transmit the virus by mechanical sap inoculation have so far yielded negative results.

Very early it was suspected that spike disease could be transmitted by one or more insect vectors, and several investigations aiming at confirming this have been carried out through the years. On this basis, some research workers have claimed that the leaf hoppers Moonia albimaculata and Jassus indicus were vectors of spike virus, but these claims have been very much argued by other workers. In any case, the apparently positive results obtained covered comparatively few plants, and even if it is possible that these insects are really the vectors, repetition of the earlier investigations and confirmation of their results would be required. Whatever the vectors may be, it seems (according to observations and field trials) that they are most active in the months April-June, with a secondary climax in October-December.

C. Control Measures so far Recommended

In order to prevent further spread of spike disease, several proposals have been put forward and some of these have also been followed in a more or less extensive manner. But unfortunately it has not so far been possible to control the disease effectively by these measures, of which the more important are mentioned below :

(a) Removal or killing of spiked sandal trees

It is obviously of great importance to eliminate sources of infection, and therefore it has been strongly recommended to remove or kill the spiked sandal trees. Such operations have also been carried out on a very large scale either by digging up the infected trees or by killing them by means of various chemicals, of which arsenicals seem to be the most effective.

(b) Removal of *Lantana camara*

Many forestry officers, and also some research workers, have pointed out the role of *Lantana camara* in the spread of sandal spike disease. *Lantana camara*, which is an exotic plant in India, was brought in from abroad over 100 years ago and has since then spread over vast areas of South India. Observations seem to indicate that spike disease of sandal trees is particularly severe and widespread in areas where the undergrowth consists mainly of *Lantana camara*, which is suspected of inducing susceptibility to spike disease, probably because it favours the possible insect vector. Removal of *Lantana camara* has, therefore, also been recommended and carried out in some places. It is, however, noteworthy that spike disease also occurs where there is no *Lantana camara*, and that in some areas where there is a great number of this plant all the sandals are apparently healthy.

(c) Selection of healthy, resistant mother-trees for seed production

In heavily spiked areas, certain sandal trees grow vigorously. Seeds from these apparently "resistant" trees have been collected and seedlings raised in the nurseries. However, this method has not proved successful, probably because the progeny did not carry the possible resistance genes of the mother-tree.

(d) Use of resistance-inducing host plants

Two types of resistance seem to exist among sandal trees, namely : (1) autogenic resistance, exhibited by certain plants and largely independent of the nature of the associated host plants; and (2) acquired resistance, which is partly induced by the associated host but is dependent also on the environmental conditions. Through pot culture experiments and field observations, attempts have been made to classify a number of host plants according to their ability to induce either resistance or susceptibility in the associated sandal plants. There is, however, some disagreement between research workers as to the conclusions of the investigations and observations leading to this classification, and it would, therefore, be advisable to repeat some of the investigations and conduct others under controlled conditions. The following grouping of some of the more important host plants has been suggested :

(i) Apparently highly resistant :

Azadirachta indica Ruta graveolens

(i) Apparently highly resistant (contd.)

<i>Bambusa arundinacea</i>	<i>Semecarpus anacardium</i>
<i>Dalbergia sissoo</i>	<i>Strychnos nux-vomica</i>
<i>Erytroxylon monogynum</i>	<i>Zizyphus jujuba</i>

(ii) Apparently resistant :

<i>Cassia siamea</i>	<i>Melia indica</i>
<i>Casuarina</i> sp.	<i>Murraya koenigii</i>
<i>Dodonaea viscosa</i>	<i>Sarostemma brevistigma</i>
<i>Ficus bengalensis</i>	

(iii) Apparently susceptible :

<i>Acacia suma</i>	<i>Dividivi</i> sp.
<i>Cassia montana</i>	<i>Ocum sanctum</i>
<i>Dalbergia latifolia</i>	

(iv) Apparently very susceptible :

<i>Acacia farnesiana</i>	<i>Melia azadirachta</i>
<i>Cajanus indicus</i>	<i>Mundulea suberosa</i>
<i>Lantana camara</i>	<i>Pongamia glabra</i>

With regard to the selection of suitable host plants, it should, however, be remembered that the different host plants' influence on the sandal tree not only varies in respect of spike resistance but also in respect of heartwood formation, growth and reproductive behaviour - all very important points to consider.

IV. PROPOSALS FOR FURTHER WORK ON SPIKE DISEASE

Before any definitive control measures against spike disease can be recommended, much more experimental work is needed. This statement must not be taken as a belittlement of the valuable investigations carried out in the past. The work already done will be highly useful when conclusions are to be drawn of the future investigations, but as some of the earlier results are still not universally accepted, it will not only be desirable but also necessary to repeat certain experiments under more controlled conditions than hitherto. In addition, some quite new investigations are advisable. Altogether, the work recommended can be dealt with under the following three headings :

- A. Diagnostic work.
- B. Transmission studies.
- C. Control measures. *

A. Diagnostic Work

(1) Indexing of various test plants

(a) Mechanical sap inoculation.

- (i) Ordinary method.
- (ii) Ordinary method - addition of phosphate buffer (pH 7).
- (iii) Dry-leaf method.

(b) Grafting methods, etc.

- (i) Bottle grafting.
- (ii) Budding.
- (iii) Leaf tissue insertion.

(c) Dodder transmission.

- (i) Use of Cuscuta gronovii.
- (ii) Use of other plant parasites.

Suggested test plants :

Sandal, citrus, peach, cherry, plum, apple, pear, Vinca rosea, Nicotiana tabacum "White Burley", N. glutinosa and N. rustica, Gomphrena globosa, Tetragonia expansa, Cucumis satibus, Chenopodium amaranticulor, Petunia hybrida and Datura stramoneum.

* The techniques of the work listed in the following are known to the staff of the Forest Research Laboratory, Bangalore.

(2) Serological Work

(a) Production of antiserum

First method :

- (i) Leaves from diseased trees are macerated (fresh or frozen) in a meat mincer or a homogenizer.
- (ii) The macerated leaves are squeezed in a cloth (cheese cloth).
- (iii) To 100 ml sap is added 2 drops of thioglycolic acid.
- (iv) Centrifuging 5,000 r.p.m./15 min. at 0°C.
- (v) Two parts of cold (-15°C) acetone is added to each part of supernatant fluid.
- (vi) Centrifuging 10,000 r.p.m./20 min. at -5°C.
- (vii) The precipitate is washed 3 times with distilled water in order to remove superfluous acetone.
- (viii) The precipitate is dissolved in a solution of 0.9 per cent NaCl (half of original sap volume).
- (ix) The solution is shaken mechanically for 20 minutes.
- (x) Centrifuging 1,500 r.p.m./5 min.
- (xi) The supernatant fluid is used for intravenous injection into rabbits.
- (xii) 10 injections should be performed (3 times per week) in doses from 2 to 8 ml.

Second method :

- (i) To 100 ml fresh expressed sap from diseased sandal leaves is added a solution consisting of :

10 ml Distilled water
300 mg NaHSO₃
300 mg KCN
830 mg Na₂HPO₄
270 mg KH₂PO₄

- (ii) Centrifuging 3,000 r.p.m./30 min.

Second method (contd.) :

- (iii) To supernatant fluid is added 50 ml cold chloroform.
- (iv) Shaking for 5 minutes.
- (v) Standing in refrigerator for 15 minutes.
- (vi) Centrifuging 3,000 r.p.m./15 min.
- (vii) Out of the 3 layers now separated the upper one is used.
- (viii) Filtration through cotton wool to remove coagulated chlorophyll.
- (ix) The solution is kept over night at 0°C.
- (x) Ultracentrifuging 60,000 x g/1 hour.
- (xi) The precipitate is washed 3 times in distilled water and afterwards dissolved in 0.9 per cent NaCl into about half of the original sap volume.
- (xii) Filtration through cotton wool.
- (xiii) Intravenous injection of rabbits (3 times weekly) with doses rising from 2 to 6 ml.

Third method :

- (i) Leaves from diseased plants are macerated in homogenizer together with distilled water (final dilution of sap 1:3).
- (ii) Squeezing through cheese cloth.
- (iii) Addition of chloroform (1/7 of sap volume) and stirring.
- (iv) Centrifuging 4,200 r.p.m./40 minutes. *
- (v) Supernatant fluid is kept in refrigerator over night.

* Rotor 823 International centrifuge.

Third method (contd.) :

- (vi) Centrifuging 4,200 r.p.m./30 min. *
- (vii) To supernatant fluid is added $(\text{NH}_4)_2\text{SO}_4$ (25 g pr ml sap).
- (viii) Centrifuging 4,200 r.p.m./60 min. *
- (ix) Dissolving of precipitate with 0.9 per cent NaCl (half volume of diluted sap).
- (x) Shaking.
- (xi) 8 per cent saturation with $(\text{NH}_4)_2\text{SO}_4$.
- (xii) Centrifuging 6,000 r.p.m./30 min.
- (xiii) 33 per cent saturation of supernatant fluid with $(\text{NH}_4)_2\text{SO}_4$.
- (xiv) Centrifuging 6,000 r.p.m./60 min.
- (xv) Precipitate is dissolved in 0.9 per cent NaCl (1/20 of original undiluted sap).
- (xvi) Dialysis in 0.9 per cent NaCl.
- (xvii) Centrifuging 2,000 r.p.m./1 min.
- (xviii) Supernatant fluid ready for injections.
- (xix) Intravenous injection of rabbits 9 times (3 succeeding days in each of 3 weeks). For every week freshly prepared virus solutions should be used.

(b) Absorption of antiserum

- (i) To 1 part of antiserum is added 4 parts of fresh sap from healthy sandal leaves.
- (ii) Solution left standing at 37°C for 2 hours.

* Rotor 823 International centrifuge.

(iii) Solution left standing at 1°C for 16 hours.

(iv) Centrifuging and discarding of precipitate.

(c) Use of antiserum

(i) All dilutions of antiserum are performed with 0.9 per cent NaCl solution.

(ii) Sap from diseased sandal leaves is clarified by heat, by dialysis or by any other suitable method - precipitate resulting from any clarifying method is removed by low-speed centrifuging.

(iii) 1/2 ml of antiserum is mixed with 1/2 ml of clarified sap in a small test tube, which is then placed in a temperature-regulated waterbath (experiments should be carried out to find the most suitable temperature - most likely temperature 40-50°C).

Purpose of diagnostic work

(i) Detection of virus-infected plants before visible symptoms appear, in order to carry out prompt removal both of infected trees and other hitherto unknown sources of infection (wild plants, etc.).

(ii) Development of quick and reliable testing methods for use in transmission studies.

(iii) Search for relationship with other, and possibly better-known, tree viruses.

B. Transmission Studies

1. Grafting transmission

Infector material :

Scions from spiked sandal trees.	With systemic expression.
Buds.	
Bark chips.	
Leaf chips.	With partial symptom expression (healthy-looking part).
Scions from spiked sandal trees.	
Buds.	
Bark chips.	
Leaf chips.	

Indicator plants :

Healthy sandal trees	-	1 year old
"	"	- 2 years old
"	"	- 3 "
"	"	- 4 "

2. Insect transmission tests

- (a) 10 minutes feeding on infector.
- (b) 10 minutes feeding on infector (2 hours pre-starving).
- (c) 3 hours feeding on infector.
- (d) 24 " " "
- (e) 3 days " " "
 - (i) 10 minutes feeding on indicator plants.
 - (ii) 3 hours " " "
 - (iii) 24 " " " "

Suggestion for insects to be used :

Jassus indicus, Moonia variabilis, Moonia albimaculata,
Myzus persicae*, Geioca phaseolis*.

* Models of specially made cages for aphid transmission tests were delivered to the Forest Research Laboratory, Bangalore, where similar cages are now being manufactured locally.

3. Soil and seed transmission tests

- (a) Untreated soil from diseased trees
 - (i) Seeds from healthy trees.
 - (ii) Seeds from diseased trees.
- (b) Heat-sterilized soil from diseased trees
 - (i) Seeds from healthy trees.
 - (ii) Seeds from diseased trees.

Frequent observation for symptoms, and, after some years, testing of individual trees for spike virus.

Purpose of transmission studies

Detection of possible means of spread in order to :

- (a) suggest any preventive measures;
- (b) develop reliable diagnostic methods.

Most emphasis should be laid upon the insect transmission studies.

C. Control Measures

1. Removal of sources of infection

When it is possible to remove all sources of infection in a certain area, it will doubtless be of considerable value in controlling further spread of disease, but, in order to do so, reliable and quick diagnostic methods have to be developed. It is worth while to bear in mind that not only trees with typical spike symptoms act as sources of infection, but also trees with masked symptoms. Further, it is likely that several other tree species, or even herbs, may act as sources of infection. When quick and reliable diagnostic methods are available, testing of the flora in severely spiked areas should therefore be performed.

2. Protection against possible vectors

With the present knowledge of spike disease and its spread, it is not possible to recommend any protective spraying method. But when future transmission studies have established with certainty which vectors may perform the spread, and when, through observations and trap experiments, it has been shown during which period these vectors are active on the sandal trees, the use of insecticides may prove to be of value, especially in plantations where spraying operations are comparatively easy to carry out. Where the sandal trees are growing in scrub jungles, spraying will naturally be rather difficult to carry out, but even here limited spraying with systemic insecticides of the infected trees (the infector plants) may under certain circumstances prove to be of value, especially if the spike virus is a long persistent one, which might be determined by the suggested transmission studies.

3. Selection and testing of apparently resistant mother-trees of sandal

For future sandal growing it would be of immense value if spike-resistant individuals of the sandal tree could be provided. Therefore, much emphasis should be laid upon the following selection and testing work:

(a) Selection

Apparently resistant, healthy-looking sandal trees growing in severely diseased areas should be selected. From each branch of these trees grafting material as well as cuttings should be taken.

(b) Testing for virus infection

The material is grafted (indexed) to healthy sandal trees (and/or other suitable test plants) growing in pots in insect-proof greenhouses. Negative transmission tests (i.e. no spike transmission) should be verified by repetition.

(c) Testing for resistance :

(i) By grafting

The cuttings from the selected mother-plants are placed in pots in insect-proof greenhouses at appropriate intervals (one year). Each plant is inoculated with spike material by grafting. After 3-4 years, the grade of resistance should be evaluated.

(ii) By insect transmission

When the vector relationship is well established, infection of the progeny (cuttings) from the selected mother-plants should be tried by insect transmission.

Control trees (susceptible sandal trees) should be inoculated with diseased material from the same sources as used under (c) (i) and (ii).

4. Selection and testing of suitable host plants

Seeds of about 20 different sandal host plants (see list on pp. 14 and 15) should be tested in the following way :

(a) Inoculation of experimental plants

Host plants associated with sandal

- (i) Inoculation of host.
- (ii) Inoculation of sandal.
- (iii) Control (non-inoculation).

Host plants growing alone

- (i) Inoculation of host.
- (ii) Control (non-inoculation).

(b) Testing of experimental plants

After an appropriate time, all the experimental plants showing no convincing symptoms should be tested by suitable methods (indexing to test plants or serological tests).

(c) Repetition of inoculations

The host plants which have shown total resistance to infection, or those having induced total resistance in the associated sandal trees, should be re-inoculated with spike in order to ascertain that resistance is complete.

5. Vegetative propagation (by cuttings in mist propagation) of virus-free resistant sandal trees and their most important host plants

If, through the testing of sandal trees and some of their host plants, it has been possible to find any spike-resistant plants, these should be propagated vegetatively in the quickest possible way, and the plants obtained should be used to substitute the spike-infected trees in the severely-affected areas. By this method, it may also be possible to provide healthy plant material for future regular plantations of sandal trees. In order to find the most suitable method of vegetative propagation, experiments should be initiated as soon as possible, and the following scheme is suggested :

Each of about 20 different host plants (see list of plants on pp. 14 and 15) and the sandal tree itself should be used in the experiments.

- (a) Cuttings from young shoots.
- (b) Cuttings from mature shoots.
 - (i) Control (untreated cuttings on outdoor beds).
 - (ii) Mist propagation.
 - (iii) Use of hormones.
 - (iv) Mist propagation and use of hormones.

6. Thermotherapeutic treatments

Some viruses have in the past years been inactivated by suitable heat treatments in specially-built incubators where the air temperature has been kept constant during the whole treatment. In order to try whether it is possible also to inactivate spike virus in the sandal plants, the following experiments are suggested :

Experimental plants: Young sandal plants growing in pots (artificially infected with spike).

Pre-treatment of all plants at 30°C during one week.

(a) Experiments:

- (i) 37°C during 1 week.
- (ii) " " 2 weeks.
- (iii) " " 3 "
- (iv) " " 4 "
- (v) " " 5 "
- (vi) " " 6 "
- (vii) Control (untreated plants).

Immediately after removal from incubators, cuttings should be taken from each treated plant.

(b) Testing of treated material

- (i) Each heat-treated plant should be tested by suitable methods soon after removal from the incubator.
- (ii) Each cutting taken from treated plants should be tested about one year, and again two years, after termination of the heat treatment.

V. RECOMMENDATIONS CONCERNING STAFF, FACILITIES AND EQUIPMENT

A. Staff

The future work on sandal spike disease is by no means a one-man job, and it is therefore very fortunate that the Indian Government has already sanctioned the recruitment of some staff for the sandal spike investigations at the Forest Research Laboratory in Bangalore. The Working Party on Sandal Spike Disease has already prepared a preliminary plan for the recruitment of this staff.

When properly staffed and equipped, the Forest Research Laboratory at Bangalore will be an ideal place for these investigations, as it is situated in the centre of the diseased areas and the Chief Research Officer of this Laboratory is very well acquainted with the problem and knows most of the people who, in the past, have been working on the spike disease. Another advantage is that the Laboratory is located next to the Indian Institute of Science and therefore can benefit very much from a close collaboration with the scientists at this Institute and make use of a variety of valuable equipment there.

The scientific staff should consist of one virologist, one entomologist, one biochemist and one forest research officer, and the technical staff should include laboratory assistants as well as some well-trained foresters (rangers) and nurserymen. The last-mentioned category of staff members - the nurserymen - is not the least important as they will be responsible for the vast number of test plants and other experimental plants needed for the investigations suggested. It may here be stressed that in several research institutes where excellent scientists are working it is not uncommon to see that many experiments are spoilt because of the lack of practical skill and knowledge of the technical staff.

Concerning the scientific staff, it is very important to appoint persons who will be able to remain at this work for a number of years in order to secure continuity in the investigations. Frequent changes of staff members - especially among the scientists - are likely to disturb and delay such work very much indeed. Whenever possible, research workers who have formerly been engaged in work on spike disease should be included in the new staff.

B. Facilities

For almost every kind of experimental work proposed, insect-proof greenhouses are absolutely necessary, and it is therefore strongly recommended that at least one such house should be built at the Forest Research Laboratory at Bangalore. The experimental greenhouses at the

Plant Virus Department of the Agricultural Research College at Poona may serve as models for those to be erected at Bangalore. A house covering about 350 m² would be suitable (sketch delivered to the Forest Research Laboratory, Bangalore), with 7 rooms to be used in the following manner :

- (1) Plants for long-term observation.
- (2) Mist propagation (sketch delivered to the Forest Research Laboratory, Bangalore).
- (3) Grafting transmissions.
- (4) Propagation of test plants (seedlings).
- (5) Working room.
- (6) Sap inoculations.
- (7) Insect transmissions (plants for observation after the transmission).

In the Laboratory building a special laboratory for plant virus work should be equipped (see under "Equipment"), and besides that comparatively small rooms should be furnished : one for propagation of insect vectors and another for insect transmission work. A special room with thermostatically-controlled temperature (range 35-40°C), and with built-in light sources for thermotherapeutic work, is also recommended.

C. Equipment

In the Forest Research Laboratory, a variety of valuable equipment is already available, but for plant virus work additional equipment will be necessary. The following list indicates the equipment still needed :

- One high-speed centrifuge (2,000-15,000 r.p.m.).
- One weight for balancing centrifuge tubes.
- Thermostatically-controlled water bath for serological tests.
- Refrigerator - zero to 4°C (for storing plant material and virus solutions).
- Binocular microscope.
- Two or three thermographs.
- Sterilizer for sterilizing soil and pots (in large quantities).
- Stericrepe bandage for grafting experiments.
- Dialysis sacks (synthetic sausage skin).
- Plastic labels.
- Small cages for aphid transmissions.
- " " " leaf hopper " .
- Insect traps (colour traps) for survey of insect fauna (model made available).

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Fig. 1 - The centre for work on sandal spike disease, the Forest Research Laboratory, Bangalore.

2 - Healthy sandal tree. Inset: Twig, flower truss and fruit from the same tree.



Fig. 3 - Spike-diseased sandal tree. Inset: Twig from the same tree.

Fig. 4 - left: Shoot from healthy sandal tree.
The three other shoots are from a
spike-diseased tree.



Fig. 5 - In upper row, leaves from healthy
sandal tree. Below, leaves from
sandal tree severely attacked by spike
disease.





